REMARKS

Claims 1- 41 are pending in the application. Claims 1- 4, 15-19, 21 and 22 are rejected. Claims 1, 2 and 21 have been amended to better clarify what Applicants believe to be the invention. No new matter has been added by way of this amendment. Accordingly, claims 1-4, 15-19, 21 and 22 remain under consideration.

Support for the amendment to claim number 2 can be found in the specification on page 30, lines 17-24 continuing on to page 31, lines 1-23.

Claim 21 has been rejected under 35 U.S.C. §112, second paragraph as being indefinite. Applicants respectfully traverse the Examiner's rejection, and have amended the claim to better clarify what Applicants regard as the invention. Support for this amendment can be found in the application on page 14, lines 10 –11; on page 25, lines 13-14; on page 38, lines 10-12; and on page 30, lines 6-8. Thus, withdrawal of the rejection under 35 U.S.C. §112, second paragraph, is respectfully requested.

Claims 1, 2, 4, 15-19, 21 and 22 have been rejected under 35 U.S.C. §103(a), as being unpatentable over Rovere et al (J. Immunol. 1998, Vol. 161, pp. 4467-4471), in view of Migita et al (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732) and Banchereau et al. (Nature, 1998, Vol. 392, pp. 245-252); and Guibinga et al. (J. Virology, 1998, Vol. 72, pp. 4601-4609) as applied to claims 1, 4, 15, 16, 17, 19, 21 and 22 and further in view of Li et al. (Transplantation 1998, Vol. 66, pp. 1387-1388) and Sehgal et al. (Clin. Biochemistry, 1998, Vol. 31, pp. 335-340). Furthermore, claims 1-3, 19, 21 and 22 have also been rejected under 35 U.S.C. §103(a) as being unpatentable over Alberts et al. (J. Exp. Med., 1998, Vol. 188, pp. 1359-1368) in view of Kurts et al., (J. Exp. Med., 1997, Vol. 186, pp. 239-245) and Steinman et al., (Immunol. Rev., 1997, Vol. 156, pp. 25-37) and Beschorner (WO 94/05323). Applicants respectfully traverse the rejection and have amended the claims to better clarify the invention. Support for this amendment can be found in the application on page 14, lines 10 –11; on page 25, lines 13-14; on page 38, lines 10-12; and on page 30, lines 6-8. Accordingly, Applicants respectfully request withdrawal of this rejection.

Claim Rejections under 35 U.S.C. §112

Claim 21 has been rejected under 35 U.S.C. §112, second paragraph as being indefinite. Applicants respectfully traverse the Examiner's rejection, and have also amended the claim to better clarify what Applicants regard as the invention. Support for this amendment can be found throughout the specification, but particularly on page 14, lines 1 –13; on page 25, lines 13-14; on page 38, lines 10-12; and on page 30, lines 6-8. In particular, page 25, lines 13-14 states:

> "Note, at the time of harvesting, the DCs demonstrate a mature phenotype based on CD83 and HLA-DR^{hi} surface expression."

Further support can be found on page 38, lines 10-12, whereby it states:

> "CD14 is a marker for macrophages which is absent on immature and mature DCs. Surface expression of CD83 is a marker for mature DCs, distinguishing it from immature DCs and macrophages.

Further support for the method described herein can be found on page 13, lines 18-23, continuing on to page 14, lines 1-13, whereby it is stated:

> "The new culturing methodology for achieving *in-vitro* tolerance has been prepared as follows: apoptotic cells are co-culture with immature DCs in the presence or absence of a maturation stimulus, mimicking events that occur in the periphery. The DCs are then harvested after 36-48 hours, and tested for their ability to activate versus tolerize influenza-specific T cell responses, an interaction which likely occurs in the draining lymph organs. Specifically, peripheral blood was obtained from normal donors in heparinized syringes and PBMCs were isolated by sedimentation over Ficoll-Hypaque (Pharmacia Biotech). T cell enriched and T cell depleted fractions were prepared by rosetting with neuraminidase-treated sheep red blood cells. Immature dendritic cells (DCs) were prepared from the T cell depleted fraction by culturing cells in the presence of granulocyte and macrophage colony-stimulating factor (GM-CSF, Immunex) and interleukin 4 (IL-4, R & D Systems) for 7 days. 1000 U/ml of GM-CSF and 500-1000 U/ml of IL4 were added to the cultures on days 0, 2 and 4. To generate mature DCs, the cultures were transferred to fresh wells on day 6-7 and monocyte conditioned media (MCM)(M. L. Albert, B. Sauter, N. Bhardwaj, *Nature* **392**, 86-9, 1998) or a mixture of 50 U/ml tumor necrosis factor-alpha (TNF-α, Endogen) and 0.1 μM prostaglandin E-2 (PGE-2, Sigma Co.) was added for an additional 1-2 days. At day 6-7, >95% of the cells were CD14-, CD83-, HLA-DRlo DCs. Post-maturation, on day 8-9, 70-95% of the cells were of the mature CD14-, CD83+, HLA-DRhi phenotype. CD4+ and CD8+ T cells were further purified to >99% purity by positive selection using the MACS column purification system (Miltenyi Biotech.)."

Claim Rejections under 35 U.S.C. §103(a)

Claims 1, 2, 4, 15-19, 21 and 22 have been rejected under 35 U.S.C. §103(a), as being unpatentable over Rovere et al (J. Immunol. 1998, Vol. 161, pp. 4467-4471), in view of Migita et al (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732) and Banchereau et al. (Nature, 1998, Vol. 392, pp. 245-252); and Guibinga et al. (J. Virology, 1998, Vol. 72, pp. 4601-4609) as applied to claims 1, 4, 15, 16, 17, 19, 21 and 22 and further in view of Li et al. (Transplantation 1998, Vol. 66, pp. 1387-1388) and Sehgal et al. (Clin. Biochemistry, 1998, Vol. 31, pp. 335-340). Furthermore, claims 1-3, 19, 21 and 22 have also been rejected under 35 U.S.C. §103(a) as being unpatentable over Alberts et al. (J. Exp. Med., 1998, Vol. 188, pp. 1359-1368) in view of Kurts et al., (J. Exp. Med., 1997, Vol. 186, pp. 239-245) and Steinman et al., (Immunol. Rev., 1997, Vol. 156, pp. 25-37) and Beschorner (WO 94/05323).

Applicants respectfully traverse the rejection and have amended the claims to better clarify the invention. Support for these amendments can be found in the application on page 13, lines 18-23, continuing on to page 14, lines 1-13; on page 25, lines 13-14; on page 38, lines 10-12; and on page 30, lines 6-8.

The Examiner has the initial burden of establishing a *prima facie* case of obviousness. A finding of obviousness under § 103 requires a determination of the scope and content of the prior art, the differences between the claimed invention and the prior art, the level of ordinary skill in the art, and whether the differences are such that the claimed subject matter as a whole would have been obvious to one of ordinary skill in the art at the time the invention was made. Graham v. Deere, 383 US 1 (1966). Obviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching or suggestion that the combination be made. In re Stencel, 828 F2d 751, 4 USPQ2d 1071 (Fed. Cir. 1987).

The invention as claimed. The claims, as amended, are drawn to methods for inducing tolerance in a mammal to an antigen comprising the steps of: isolating peripheral blood mononuclear cells (PBMC) from a whole blood sample from said mammal; isolating dendritic cells from said PBMC; exposing said dendritic cells *ex vivo* to apoptotic cells expressing said antigen in the presence of at least one dendritic cell

maturation stimulatory molecule and in the absence of effective CD4+ T cell help, wherein said dendritic cells upon exposure to said dendritic cell maturation stimulatory molecule are characterized as having the phenotype $CD14^-$, $CD83^+$ and $HLA\text{-}DR^{hi}$; and introducing the dendritic cells into said mammal; wherein said dendritic cells induce apoptosis of antigen-specific CD8+ T cells in said mammal resulting in tolerance to said antigen. The dependent claims are drawn to particular dendritic cell maturation factors including PGE2, TNF-alpha, lipopolysaccharide, monocyte conditioned medium, CpG-DNA, or any combination thereof. Additional dependent claims are drawn to methods for exclusion of effective CD4+ T cell help including exclusion of CD4+ T cells from the culture or whereby the absence of effective CD4+ T cell help is achieved by including at least one agent that inhibits or eliminates effective CD4+ T cell help. The agent which inhibits or eliminates effective CD4 T cell help inhibits signalling consequent to dendritic cell-CD4 T cell engagement. Such an agent is selected from a FKBP antagonist and a TOR antagonist. The FKBP antagonist is tacrolimus. The TOR antagonist is rapamycin. The antigen is a tumor antigen, a viral antigen, a self-antigen or a transplant antigen. The dendritic cells are infused into the mammal after the dendritic cells mature and exhibit the phenotype $CD14^-$, $CD83^+$ and $HLA\text{-}DR^{hi}$ and the mammal is a human.

The Rovere et al. reference as a whole. Rovere et al. teach that dendritic cells control their own maturation by releasing maturation factors, when challenged with an excess of apoptotic cells.

Rovere et al. **do not teach the methods of the present invention**. In particular, Rovere et al. do not teach a method for inducing tolerance to an antigen by having the dendritic cell phagocytose apoptotic cells containing the antigen to which tolerance is desired in the presence of a dendritic cell maturation factor in the absence of CD4+ T cell help, which then leads to maturation of the dendritic cells such that the mature dendritic cells now exhibit high levels of surface expression of CD83 and $HLA\text{-}DR^{hi}$, but do not express CD14. Furthermore, Rovere et al. do not teach that introducing the dendritic cells having these markers into a mammal results in induction of apoptosis of antigen-specific CD8+ T cells in the mammal resulting in tolerance to the antigen. In addition, Rovere et al. **do not contemplate the use of agents that inhibit or eliminate effective**

CD4+ T cell help, such as FK506 or rapamycin, which inhibit or eliminate effective CD4+ T cell help by inhibiting signalling consequent to dendritic cell-CD4+ T cell engagement.

Banchereau et al. reference as a whole. The Banchereau et al. reference is a general review article which teaches that dendritic cells are involved in the induction of peripheral tolerance.

Banchereau et al. do not teach the methods of the present invention. In particular, Banchereau et al. **do not teach that the presentation of antigen by apoptotic cells to the dendritic cell with a dendritic cell maturation factor in the absence of CD4+ T cell help results in induction of tolerance**. In particular, Banchereau et al. **do not teach** that following exposure of the dendritic cells to a maturation stimulus in the absence of CD4+ T cell help results in **generation of a population of dendritic cells expressing high levels of surface expression of $CD83^+$ and $HLA\text{-}DR^{hi,}$ but which are $CD14^-$.** Banchereau et al. also do not teach that introducing the dendritic cells having these markers into a mammal results in induction of apoptosis of antigen-specific CD8+ T cells in the mammal resulting in tolerance to the antigen. In addition, Banchereau et al. **do not contemplate the use of agents that inhibit or eliminate effective CD4+ T cell help, such as FK506 or rapamycin**, which inhibit or eliminate effective CD4+ T cell help by inhibiting signalling consequent to dendritic cell-CD4+ T cell engagement.

Migita et al. reference as a whole. Migita et al. teach that FK506 enhances the apoptotic effect of anti-CD3 antibody. FK506 by itself does not induce apoptosis.

Migita et al. do not teach the methods of the present invention. In particular, Migita et al. do not teach that the presentation of antigen by apoptotic cells to the dendritic cell with a dendritic cell maturation factor in the absence of CD4+ T cell help results in induction of tolerance. In particular, Migita et al. do not teach that following exposure of the dendritic cells to a maturation stimulus in the absence of CD4+ T cell help results in generation of a population of dendritic cells expressing high levels of surface expression of $CD83^+$ and $HLA\text{-}DR^{hi,}$ but which are $CD14^-$. Furthermore, Migita et al. do not teach that the agent of the present invention, eg. FK506 or rapamycin, which

inhibits or eliminates effective CD4+ T cell help, does so by inhibiting signalling **consequent to dendritic cell-CD4+ T cell engagement**.

Guibinga et al. reference as a whole. Guibinga et al. teach the use of FK506 to facilitate therapy by gene transfer by Adenovirus vectors. In particular, Guibinga et al. teach the use of FK506 in combination with CTLA4Ig to abrogate the immune response against Adenovirus proteins.

Guibinga et al.do not teach or disclose the methods of the present invention. More particularly, Guibinga et al. **do not teach** that presentation of antigen for which tolerance is desired via apoptotic cells to dendritic cells, in the presence of a dendritic cell maturation factor, but in the absence of T cell help using agents such as FK506, results in **generation of dendritic cells having a phenotype of $CD14^-$, $CD83^+$, and $HLA\text{-}DR^{hi}$, which when administered to a subject results in apoptosis of antigen specific T cells.**

The Li et al. reference as a whole. Li et al. teach that tolerance to an allograft may be induced using **rapamycin as adjunct therapy with a co-stimulation blockade** (anti-CD40 ligand plus CTLA-4Ig). Furthermore, **Li et al. teach that rapamycin blocks IL-2 induced proliferation, but not apoptotic signals.**

Li et al. do not teach the methods of the present invention. In particular, Li et al. do not teach the presentation of antigen for which tolerance is desired via apoptotic cells to dendritic cells in the presence of a dendritic cell maturation factor, but in the absence of CD4+ T cell help. More particularly, Li et al. do not teach the methods of the present invention which result in the generation of a population of $CD14^-$, $CD83^+$, $HLA\text{-}DR^{hi}$ dendritic cells, which when administered to a human subject result in apoptosis of antigen specific T cells. Furthermore, Li et al. do not teach that the agents of the present invention, eg. FK506 or rapamycin, which inhibit or eliminate effective CD4+ T cell help, do so by **inhibiting signalling consequent to dendritic cell-CD4+ T cell engagement**.

The Sehgal reference as a whole. Sehgal teaches that rapamycin complexes with the immunophilin FKBP to produce the mammalian inhibitor of rapamycin complex which

blocks the IL-2 mediated signal transduction pathway that prevents cell cycle progression from G1 to S phase in T cells.

Sehgal does not teach the methods of the present invention. In particular, Sehgal does not teach the presentation of antigen for which tolerance is desired via apoptotic cells to dendritic cells in the presence of a dendritic cell maturation factor, but in the absence of CD4+ T cell help. More particularly, Sehgal does not teach the methods of the present invention which result in the generation of a population of $CD14^-$, $CD83^+$, $HLA\text{-}DR^{hi}$ dendritic cells, which when administered to a human subject result in apoptosis of antigen specific T cells. Furthermore, Sehgal does not teach that the agents of the present invention, eg. FK506 or rapamycin, which inhibit or eliminate effective CD4+ T cell help, do so by **inhibiting signalling consequent to dendritic cell-CD4+ T cell engagement**. In addition, **the target of RAPA:FKBP is distinct from calcineurin**, unlike FK506, which when complexed with its respective immunophilin inhibits calcineurin, which is required for early T cell activation.

The Albert et al. reference as a whole. Albert et al, teach that dendritic cells phagocytose apoptotic cells and cross present antigen in this manner to cytotoxic T lymphocytes (CTLs). Furthermore, Albert et al. teach the isolation of dendritic cells from peripheral blood and the use of monocyte conditioned medium as a maturation factor for the dendritic cells for induction of antigen specific CTLs.

Albert et al. do not teach the methods of the present invention. In particular, Albert et al. **do not teach that the absence of CD4+ T cell help, or the inhibition of CD4+ T cell help through the use of inhibitors of signalling consequent to dendritic cell-CD4+ T cell engagement is a requirement in conjunction with the generation of $CD14^-$, $CD83^+$, and $HLA\text{-}DR^{hi}$ dendritic cells following addition of maturation factors** using the methods described in the present application for tolerance induction. In addition, Albert et al. do not teach the inhibition of signalling using agents such as those described herein, such as FK506 or rapamycin.

The Kurts et al. reference as a whole. Kurts et al. teach that tolerance induced by cross-presented self-antigens relates to a lack of CD4+ T cell help.

Kurts et al. do not teach the methods of the present invention for inducing tolerance. In particular, Kurts et al **do not teach the presentation of antigen for which tolerance is desired via apoptotic cells to dendritic cells in the presence of a dendritic cell maturation factor** in the absence of CD4+ T cell help. In addition, Kurts et al. **do not contemplate the use of agents that inhibit or eliminate effective CD4+ T cell help, such as FK506 or rapamycin**, which inhibit or eliminate effective CD4+ T cell help by inhibiting signalling consequent to dendritic cell-CD4+ T cell engagement. More particularly, Kurts et al. do not teach the methods of the present invention which result in the generation of a population of $CD14^-$, $CD83^+$, $HLA\text{-}DR^{hi}$ dendritic cells, which when administered to a human subject result in apoptosis of antigen specific T cells.

The Steinman et al reference as a whole. Steinman et al. teach that dendritic cells in the T cell areas may function to maintain peripheral tolerance to self-antigens by deleting CD4+ T cells via the fas-1 mediated pathway.

Steinman et al. do not teach the methods of the present invention. In particular, Steinman et al. **do not teach or contemplate the presentation of antigen for which tolerance is desired via apoptotic cells to dendritic cells in the presence of a maturation factor for dendritic cells while at the same time eliminating CD4+ T cell help**. In addition, Steinman et al. **do not contemplate the use of agents that inhibit or eliminate effective CD4+ T cell help, such as FK506 or rapamycin**, which inhibit or eliminate effective CD4+ T cell help by inhibiting signalling consequent to dendritic cell-CD4+ T cell engagement. More particularly, Steinman et al. **do not teach** the methods of the present invention which result in the **generation of a population of $CD14^-$, $CD83^+$, $HLA\text{-}DR^{hi}$ dendritic cells**, which when administered to a human subject result in apoptosis of antigen specific T cells.

The Beschorner reference as a whole. Beschorner teaches a method for the induction of antigen specific immune tolerance by depleting resident thymic antigen presenting cells and repopulation of the thymus with new antigen presenting cells containing the antigen for tolerance. The method further contemplates the use of hGH, hIGF-1 or related agents after infusion of antigen presenting cells.

Beschorner does not teach the methods of the present invention. In particular, Beschorner **does not teach or suggest use of an ex vivo system** such as that described herein **for production of a population of $CD14^-$, $CD83^+$, $HLA\text{-}DR^{hi}$ dendritic cells**, generated in response to exposure to antigen presented via apoptotic cells in the presence of a dendritic cell maturation factor and in the absence of CD4+ T cell help, or under conditions whereby effective CD4+ T cell help is inhibited, such as through use of the agents described herein, eg. FK506 or rapamycin.

The analysis under § 103(a). The references noted herein do not teach the methods disclosed in the present application for tolerance induction ex vivo. Moreover, since the methods described in the present application for tolerance induction were unknown at the time of the references cited, it was not possible to predict the steps and conditions necessary to optimize induction of antigen specific tolerance. Moreover, it was not until Applicants' present invention that the precise steps involved in tolerance induction by presenting antigen to dendritic cells via apoptotic cells in the presence of dendritic cell maturation factors, but in the absence of CD4+ T cell help, which then resulted in the generation of dendritic cells bearing the phenotype $CD14^-$, $CD83^+$, $HLA\text{-}DR^{hi}$, which upon transfer to a subject resulted in apoptosis of antigen specific CD8+ T cells, were identified. Furthermore, it was not until the time of Applicants' own research that it was realized that the absence of CD4+ T cell help could be substituted by the use of inhibitors of signalling **consequent to dendritic cell-CD4+ T cell engagement**, such as with FK506 or rapamycin.

Thus, the connection between the work done by Rovere et al (J. Immunol. 1998, Vol. 161, pp. 4467-4471), in view of Migita et al (J. Clin. Investigation, 1995, Vol. 96, pp. 727-732) and Banchereau et al. (Nature, 1998, Vol. 392, pp. 245-252); and Guibinga et al. (J. Virology, 1998, Vol. 72, pp. 4601-4609) as applied to claims 1, 4, 15, 16, 17, 19, 21 and 22 could not have been made in light of Li et al. (Transplantation 1998, Vol. 66, pp. 1387-1388) and Sehgal et al. (Clin. Biochemistry, 1998, Vol. 31, pp. 335-340), since the precise steps for optimization of ex vivo tolerance induction were not identified until the time of the present invention.

Likewise, the connection between Alberts et al. (J. Exp. Med., 1998, Vol. 188, pp. 1359-1368) in view of Kurts et al., (J. Exp. Med., 1997, Vol. 186, pp. 239-245) and Steinman et al., (Immunol. Rev., 1997, Vol. 156, pp. 25-37) and Beschorner (WO 94/05323) as related to claims 1-3, 19, 21 and 22 could also not have been made since the conditions for induction of ex vivo tolerance were not perfected until the time of the present invention by Applicants.

It is Applicant(s) contention that Examiner has tried to reconstruct Applicants' invention using hindsight reconstruction, which is impermissible.

In light of the foregoing claim amendments and arguments, Applicants respectfully request withdrawal of the rejection.

Fees

No fees are believed to be necessitated by the instant response. However, should this be in error, authorization is hereby given to charge Deposit Account No. 11-1153 for any underpayment, or to credit any overpayments.

Conclusion

Withdrawal of the rejections is respectfully requested. If a discussion with the undersigned will be of assistance in resolving any remaining issues, the Examiner is invited to telephone the undersigned at (201) 487-5800, ext. 118, to effect a resolution.

Respectfully submitted,

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